

## METHOD TO DETECT IgE

### Field of the Invention

The present invention relates to a novel method to detect epsilon immunoglobulin (IgE). The present invention also includes novel kits to detect IgE as well as methods to produce the detection reagent.

### Background of the Invention

Diagnosis of disease and determination of treatment efficacy are important tools in medicine. In particular, detection of IgE production in an animal can be indicative of disease. Such diseases include, for example, allergy, atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia. In addition, detection of IgE production in an animal following a treatment is indicative of the efficacy of the treatment, such as when using treatments intended to disrupt IgE production.

Until the discovery of the present invention, detection of IgE in samples obtained from non-human animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to detect IgE in IgE-containing compositions. In particular, antibodies that bind selectively to epsilon idiotype antibodies (i.e., anti-IgE antibodies) have been used to detect IgE. These anti-IgE antibodies, however, can cross-react with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a Fc epsilon receptor (Fc<sub>ε</sub>R) molecule to detect the presence of IgE in a putative IgE-containing composition. A Fc<sub>ε</sub>R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a Fc<sub>ε</sub>R molecule can bind to an IgE with more

specificity (i.e., less idotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE binding antibodies.

Lowenthal et al., 1993, *Annals of Allergy* 71:481-484, dog serum can transfer cutaneous reactivity to a human. While it is possible that Lowenthal et al. properly teach the binding of human Fc<sub>ε</sub>R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the transfer of cutaneous reactivity. As such, a skilled artisan would conclude that the transfer of cutaneous reactivity taught by Lowenthal et al. could be due to a variety of different molecular interactions and that the conclusion drawn by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified human Fc<sub>ε</sub>R to detect canine IgE. The subunits of human Fc<sub>ε</sub>R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Patent No. 4,962,035, to Leder et al., issued October 9, 1990, discloses human Fc<sub>ε</sub>R but does not disclose the use of such a human Fc<sub>ε</sub>R to detect human or non-human IgE. The use of purified human Fc<sub>ε</sub>R avoids complications presented by use of Fc<sub>ε</sub>R bound to a cell, such as non-specific binding of the Fc<sub>ε</sub>R-bearing cell due to additional molecules present on the cell membrane. That purified human Fc<sub>ε</sub>R detects non-human IgE is unexpected because inter-species binding between a Fc<sub>ε</sub>R and an IgE is not predictable. For example, human Fc<sub>ε</sub>R binds to rat IgE but rat Fc<sub>ε</sub>R does not bind to human IgE.

The high affinity Fc<sub>ε</sub>R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the alpha chain (Kochan et al., *Nucleic Acids Res.* 16:3584, 1988; Shimizu et al., *Proc. Natl. Acad. Sci. USA* 85:1907-1911, 1988; and Pang et al., *J. Immunol.* 151:6166-6174, 1993); the beta chain

(Kuster et al., *J. Biol. Chem.* 267:12782-12787, 1992); and the gamma chain (Kuster et al., *J. Biol. Chem.* 265:6448-6452, 1990).

Thus, methods and kits are needed in the art that will provide specific detection of non-human IgE.

5

### Summary of the Invention

The present invention includes detection methods and kits that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated human  $\text{Fc}_\epsilon\text{R}$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) molecule with a putative IgE-containing composition under conditions suitable for formation of a  $\text{Fc}_\epsilon\text{R}$  molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the  $\text{Fc}_\epsilon\text{R}$  molecule:IgE complex, the presence of the  $\text{Fc}_\epsilon\text{R}$  molecule:IgE complex indicating the presence of IgE. A preferred  $\text{Fc}_\epsilon\text{R}$  molecule in which a carbohydrate group of the  $\text{Fc}_\epsilon\text{R}$  molecule is conjugated to biotin.

15

Another embodiment of the present invention is a method to detect IgE comprising: (a) contacting a recombinant cell with a putative IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a human  $\text{Fc}_\epsilon\text{R}$  molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred recombinant cell includes a RBL-h $\text{Fc}_\epsilon\text{R}$  cell.

20

Another embodiment of the present invention is a method to detect flea allergy dermatitis comprising: (a) immobilizing a flea allergen on a substrate; (b) contacting the flea allergen with a putative IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate; (c) removing non-bound material from the substrate under conditions that retain antigen:IgE complex binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a  $\text{Fc}_\epsilon\text{R}$  molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

The present invention also includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a human  $\text{Fc}_\epsilon$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a human  $\text{Fc}_\epsilon$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a human  $\text{Fc}_\epsilon$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) molecule and a flea allergen.

Another embodiment of the present invention is an isolated human  $\text{Fc}_\epsilon$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) alpha chain protein, in which a carbohydrate group of the  $\text{Fc}_\epsilon\text{R}$  alpha chain protein is conjugated to biotin. A preferred  $\text{Fc}_\epsilon\text{R}$  alpha chain protein comprises  $\text{PhFc}_\epsilon\text{R}\alpha_{172}\text{-BIOT}$ .

#### Brief Description of the Figures

Fig. 1 depicts ELISA results using biotinylated alpha chain of human  $\text{Fc}_\epsilon\text{R}$  to detect canine IgE antibodies.

Fig. 2 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect plant allergen-specific canine IgE antibodies.

Fig. 3 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect human or canine IgE antibodies.

5            Fig. 4 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect flea allergen-specific canine IgE antibodies.

Fig. 5 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

10           Fig. 6 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect flea saliva-specific canine IgE antibodies.

Fig. 7 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect heartworm antigen-specific feline IgE antibodies.

Fig. 8 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect heartworm antigen-specific feline IgE antibodies.

15           Fig. 9 depicts ELISA results using biotinylated alpha chain of human  $Fc_\epsilon R$  to detect antigen-specific equine IgE antibodies.

Fig. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of human  $Fc_\epsilon R$  to detect canine IgE antibodies in sera from heartworm-infected dogs.

20           Fig. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of human  $Fc_\epsilon R$  to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

## Detailed Description of the Invention

The present invention relates to the discovery that purified high affinity human Fc epsilon receptor (i.e., Fc<sub>ε</sub>RI; referred to herein as Fc<sub>ε</sub>R) can be used in certain non-human (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of  
5 human Fc<sub>ε</sub>R to detect non-human IgE is unexpected because canine, feline and equine immune systems are different from the human immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

One embodiment of the present invention is a method to detect a non-human IgE  
10 using an isolated human Fc<sub>ε</sub>R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. It is also to be  
15 noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds.

According to the present invention, an isolated, or biologically pure, Fc<sub>ε</sub>R  
20 molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated human Fc<sub>ε</sub>R molecule of the present invention

can be obtained from its natural source (e.g., from a human mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

A  $\text{Fc}_\epsilon\text{R}$  molecule (also referred to herein as  $\text{Fc}_\epsilon\text{R}$  or  $\text{Fc}_\epsilon\text{R}$  protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention can comprise a complete  $\text{Fc}_\epsilon\text{R}$  (i.e., alpha, beta and gamma  $\text{Fc}_\epsilon\text{R}$  chains), an alpha  $\text{Fc}_\epsilon\text{R}$  chain (also referred to herein as  $\text{Fc}_\epsilon\text{R}$   $\alpha$  chain) or portions thereof. Preferably, a  $\text{Fc}_\epsilon\text{R}$  molecule comprises at least a portion of a  $\text{Fc}_\epsilon\text{R}$   $\alpha$  chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention binds to IgE with an affinity of about  $K_A \approx 10^8$ , more preferably with an affinity of about  $K_A \approx 10^9$  and even more preferably with an affinity of about  $K_A \approx 10^{10}$ .

An isolated  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the  $\text{Fc}_\epsilon\text{R}$  molecule's ability to form an immunocomplex with an IgE. Examples of  $\text{Fc}_\epsilon\text{R}$  homologs include  $\text{Fc}_\epsilon\text{R}$  proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of forming an immunocomplex with an IgE.

$\text{Fc}_\epsilon\text{R}$  homologs can be the result of natural allelic variation or natural mutation.  $\text{Fc}_\epsilon\text{R}$  homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to

the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

According to the present invention, a human  $Fc_\epsilon R$   $\alpha$  chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length  $Fc_\epsilon R$   $\alpha$  chain protein represented herein as SEQ ID NO:1, the portion at least encoding the IgE binding site of the  $Fc_\epsilon R$   $\alpha$  chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:1 and the complementary non-coding strand ( the nucleic acid sequence of which can be readily determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as  $Fc_\epsilon R$  nucleic acid molecule  $nhFc_\epsilon R\alpha_{1198}$ . Translation of SEQ ID NO:1 suggests that nucleic acid molecule  $nhFc_\epsilon R\alpha_{1198}$  encodes a full-length  $Fc_\epsilon R$   $\alpha$  chain protein of about 257 amino acids, referred to herein as  $PhFc_\epsilon R\alpha_{257}$ , represented by SEQ ID NO:2, assuming an open reading frame having an initiation (start) codon spanning from about nucleotide 107 through about nucleotide 109 of SEQ ID NO:1 and a termination (stop) codon spanning from about nucleotide 878 through about nucleotide 880 of SEQ ID NO:1. The coding region encoding  $PhFc_\epsilon R\alpha_{257}$ , including the stop codon, is represented by nucleic acid molecule  $nhFc_\epsilon R\alpha_{774}$ , having a coding strand with the nucleic acid sequence represented herein as SEQ ID NO:4. SEQ ID NO:1 encodes a signal peptide of about 25 amino acids as well as a mature protein of about 232 amino acids, denoted herein as  $PhFc_\epsilon R\alpha_{232}$ , the amino acid sequence of which is represented herein as SEQ ID NO:6. The nucleic acid molecule encoding the apparent mature protein is referred to as  $nhFc_\epsilon R\alpha_{699}$ , the nucleic acid sequence of the coding strand of which is denoted herein as SEQ ID NO:7. SEQ ID NO:1 also encodes a hydrophobic



transmembrane domain and a cytoplasmic tail which as a group extend from about amino acid 205 to about amino acid 257 of SEQ ID NO:2. Knowledge of these nucleic acid and amino acid sequences allows one skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a  $\text{Fc}_\epsilon\text{R}$   $\alpha$  chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g.,  $\text{PhFc}_\epsilon\text{R}\alpha_{197}$  and  $\text{PhFc}_\epsilon\text{R}\alpha_{172}$ . Preferred  $\text{Fc}_\epsilon\text{R}$  molecules include  $\text{PhFc}_\epsilon\text{R}\alpha_{257}$ ,  $\text{PhFc}_\epsilon\text{R}\alpha_{197}$ ,  $\text{PhFc}_\epsilon\text{R}\alpha_{232}$  and  $\text{PhFc}_\epsilon\text{R}\alpha_{172}$ . Preferred nucleic acid molecules to encode a  $\text{Fc}_\epsilon\text{R}$  molecules include  $\text{nhFc}_\epsilon\text{R}\alpha_{774}$ ,  $\text{nhFc}_\epsilon\text{R}\alpha_{1198}$ ,  $\text{nhFc}_\epsilon\text{R}\alpha_{612}$ ,  $\text{nhFc}_\epsilon\text{R}\alpha_{591}$ ,  $\text{nhFc}_\epsilon\text{R}\alpha_{699}$  and/or  $\text{nhFc}_\epsilon\text{R}\alpha_{516}$ .

Isolated  $\text{Fc}_\epsilon\text{R}$  molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell that is capable of expressing the protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred

Fc<sub>ε</sub>R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention include nhFc<sub>ε</sub>Rα<sub>774</sub>, nhFc<sub>ε</sub>Rα<sub>1198</sub>, nhFc<sub>ε</sub>Rα<sub>612</sub>, nhFc<sub>ε</sub>Rα<sub>591</sub>, nhFc<sub>ε</sub>Rα<sub>699</sub> and/or nhFc<sub>ε</sub>Rα<sub>516</sub>.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a Fc<sub>ε</sub>R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including protozoa and ectoparasite), insect, other animal and plant cells.

Preferably, a recombinant cell is transfected with a recombinant molecule of the present invention is a molecule that can include at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc<sub>ε</sub>Rα<sub>612</sub>. Details regarding the production of Fc<sub>ε</sub>R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes *Trichoplusia ni*-pVL-nhFc<sub>ε</sub>Rα<sub>612</sub>.

A  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention can include chimeric molecules comprising a portion of a  $\text{Fc}_\epsilon\text{R}$  molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the  $\text{Fc}_\epsilon\text{R}$  portion binds to IgE in essentially the same manner as a  $\text{Fc}_\epsilon\text{R}$  molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin molecule.

A  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention can be contained in a formulation, herein referred to as a  $\text{Fc}_\epsilon\text{R}$  formulation. For example, a  $\text{Fc}_\epsilon\text{R}$  can be combined with a buffer in which the  $\text{Fc}_\epsilon\text{R}$  is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a  $\text{Fc}_\epsilon\text{R}$  can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with  $\text{Fc}_\epsilon\text{R}$  or conjugated (i.e., attached) to  $\text{Fc}_\epsilon\text{R}$  in such a manner as to not substantially interfere with the ability of the  $\text{Fc}_\epsilon\text{R}$  to selectively bind to IgE.

A  $\text{Fc}_\epsilon\text{R}$  of the present invention can be bound to the surface of a cell expressing the  $\text{Fc}_\epsilon\text{R}$ . A preferred  $\text{Fc}_\epsilon\text{R}$ -bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a human  $\text{Fc}_\epsilon\text{R}$  alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule that encodes at least one of the following proteins:  $\text{PhFc}_\epsilon\text{R}\alpha_{257}$  and  $\text{PhFc}_\epsilon\text{R}\alpha_{232}$ . An even

more preferred recombinant cell expresses a nucleic acid molecule including nhFc<sub>ε</sub>Rα<sub>612</sub>, nhFc<sub>ε</sub>Rα<sub>591</sub>, nhFc<sub>ε</sub>Rα<sub>699</sub> and/or nhFc<sub>ε</sub>Rα<sub>516</sub> with a recombinant cell expressing a nucleic acid molecule comprising a nucleic acid sequence including SEQ ID NO:1 or SEQ ID NO:4, or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:4, being even more preferred. An even more preferred recombinant cell is a RBL-hFc<sub>ε</sub>R cell.

In addition, a Fc<sub>ε</sub>R formulation of the present invention can include not only a Fc<sub>ε</sub>R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to any molecule capable of being selectively bound by an antibody. As used herein, specific binding of a first molecule to a second molecule refers to the ability of the first molecule to preferentially bind (e.g., having higher affinity higher avidity) to the second molecule when compared to the ability of a first molecule to bind to a third molecule. The first molecule need not necessarily be the natural ligand of the second molecule. Examples of such antibodies include, but are not limited to, antibodies that bind selectively to the constant region of an IgE heavy (i.e., anti-IgE isotype antibody) or antibodies that bind selectively to an IgE having a specific antigen specificity (i.e., anti-IgE idiotypic antibody). Examples of such antigens include any antigen known to induce the production of IgE. Preferred antigens include allergens and parasite antigens. Allergens of the present invention are preferably derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybeans, rice, eggs, milk, cheese, bovines (or cattle), poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs or ticks. A suitable flea allergen includes an allergen derived from a flea, in particular flea saliva antigen. A preferred flea allergen includes a

flea saliva antigen Preferred flea saliva antigens include antigens such as those disclosed in PCT Patent Publication No. WO 96/11271, published April 18, 1996, by Frank et al. (this publication is incorporated by reference herein in its entirety), with flea saliva products and flea saliva proteins being particularly preferred. According to the present invention, a flea saliva protein includes a protein produced by recombinant DNA methods, as well as proteins isolated by other methods disclosed in PCT Patent Publication No. WO 96/11271.

Preferred general allergens include those derived from grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb's Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach, *Dermataphagoides*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pullularia*, *Rhizopus* and/or *Tricophyton*. More preferred general allergens include those derived from Johnson Grass, Kentucky Blue Grass, Meadow Fescue, Orchard Grass, Perennial Rye Grass, Redtop Grass, Timothy Grass, Bermuda Grass, Brome Grass, Curly Dock, English Plantain, Mexican Firebush, Lamb's Quarters, Rough Pigweed Short Ragweed, Wormwood Sage, American Elm, Common Cocklebur, Box Elder, Black Walnut, Eastern Cottonwood, Green Ash, River Birch, Red Cedar, Red Oak, Red Mulberry, Cockroach, *Dermataphagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Fusarium vasinfectum*, *Helminthosporium sativum*, *Mucor recemosus*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans* and/or *Tricophyton* spp. Preferred parasite antigens include, but are not limited to, helminth antigens, in particular heartworm antigens, such as Di33 (described in U.S. Patent Application Serial No. 08/715,628, filed

September 18, 1996, to Grieve et al.). The term "derived from" refers to a natural allergen of such plants or organisms (i.e., an allergen directly isolated from such plants or organisms), as well as, non-natural allergens of such plants or organisms that possess at least one epitope capable of eliciting an immune response against an allergen (e.g.,  
5 produced using recombinant DNA technology or by chemical synthesis).

The present invention also includes human  $\text{Fc}_\epsilon\text{R}$  mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetoep" refers to any compound that is able to mimic the ability of a  $\text{Fc}_\epsilon\text{R}$  molecule to bind to IgE. A mimetoep can be a peptide that has been modified to decrease its susceptibility to  
10 degradation but that still retains IgE-binding activity. Other examples of mimetopes include, but are not limited to, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimetoep can be obtained by, for example, screening libraries of synthetic compounds  
15 for compounds capable of binding to IgE. A mimetoep can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer  
20 modeling. The predicted mimetoep structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetoep from a natural source. Specific examples of  $\text{Fc}_\epsilon\text{R}$  mimetopes include anti-idiotypic antibodies,

oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology.

One embodiment of the present invention is a method to detect non-human IgE which includes the steps of: (a) contacting an isolated human  $\text{Fc}_\epsilon\text{R}$  (Fc $_\epsilon$ R) molecule with a putative IgE-containing composition under conditions suitable for formation of an Fc $_\epsilon$ R molecule:IgE complex; and (b) detecting levels of IgE by detecting said Fc $_\epsilon$ R molecule:IgE complex. Presence of such a Fc $_\epsilon$ R molecule:IgE complex indicates that the animal is producing IgE. Preferred non-human IgE to detect using a human Fc $_\epsilon$ R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a Fc $_\epsilon$ R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an IgE is heat labile when incubated at about 56°C for about 4 hours. Without being bound by theory, Applicants believe that heat labile forms of IgE bind to certain allergens and non-heat labile forms of IgE bind to other types of allergens. As such, detection of heat labile IgE compared with non-heat labile IgE can be used to discriminate between allergen sensitivities. For example, Applicants believe that IgE antibodies that bind to certain flea allergens and heartworm allergens are heat labile while IgE antibodies that bind to certain plant allergens are not heat labile. Thus, the presence of non-heat labile IgE can indicate that an animal is sensitive to certain plant allergens but not to certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a human Fc $_\epsilon$ R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a Fc $_\epsilon$ R molecule of the present invention may

be useful for detecting molecules bound by the Fc<sub>ε</sub>R molecule but not identical to a known IgE.

As used herein, canine refers to any member of the dog family, including domestic dogs, wild dogs and zoo dogs. Examples of dogs include, but are not limited to, domestic dogs, wild dogs, foxes, wolves, jackals and coyotes. As used herein, a feline refers to any member of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs, and servals. As used herein, equine refers to any member of the horse family, including horses, donkeys, mules and zebras.

As used herein, the term “contacting” refers to combining or mixing, in this case a putative IgE-containing composition with a human Fc<sub>ε</sub>R molecule. Formation of a complex between a Fc<sub>ε</sub>R and an IgE refers to the ability of the Fc<sub>ε</sub>R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a Fc<sub>ε</sub>R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a Fc<sub>ε</sub>R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989, the reference Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety.



As used herein, the term “detecting complex formation” refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between Fc<sub>ε</sub>R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the art (see, for example, Sambrook et al. *ibid.*), examples of which are disclosed herein.

In one embodiment, a putative IgE-containing composition of the present method includes a biological sample from an animal. A suitable biological sample includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Such a composition of the present method can, but need not be, pretreated to remove at least some of the non-IgE isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as Protein G, to remove IgG antibodies and/or affinity purifying IgE antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A. In another embodiment, a composition includes collected bodily fluid that is pretreated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using ammonium sulfate. A preferred composition of the present method is serum.

In another embodiment, a composition of the present method includes an IgE-producing cell. Such a cell can have IgE bound to the surface of the cell and/or can secrete IgE. Examples of such cells include basophil cells and myeloma cells. IgE can be bound to the surface of a cell either directly to the membrane of a cells or bound to a molecule (e.g., an antigen) bound to the surface of the cell.

A complex can be detected in a variety of ways including, but not limited to use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a detectable marker. In other assays, conjugation (i.e., attachment) of a detectable marker to the  $Fc_\epsilon R$  or to a reagent that selectively binds to the  $Fc_\epsilon R$  or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a

peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a  $Fc_\epsilon R$ . Preferably a carbohydrate group of the  $Fc_\epsilon R$  alpha chain is conjugated to biotin. A preferred  $Fc_\epsilon R$  molecule conjugated to biotin comprises  $PhFc_\epsilon R\alpha_{172}$ -BIOT (the production of which is described in the Examples section).

In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a  $Fc_\epsilon R$  molecule that is conjugated to a detectable marker. A suitable detectable marker to conjugate to a  $Fc_\epsilon R$  molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A detectable marker is conjugated to a  $Fc_\epsilon R$  molecule or a reagent in such a manner as not to block the ability of the  $Fc_\epsilon R$  or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a  $Fc_\epsilon R$  is conjugated to biotin.

In another embodiment, a  $Fc_\epsilon R$  molecule:IgE complex is detected by contacting a putative IgE-containing composition with a  $Fc_\epsilon R$  molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the  $Fc_\epsilon R$  molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a  $Fc_\epsilon R$  molecule, an antigen, an antibody and a lectin, depending upon which portion of the  $Fc_\epsilon R$  molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- $Fc_\epsilon R$  antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a  $Fc_\epsilon R$  molecule of the present invention

produced in insect cells. An indicator molecule itself can be attached to a detectable marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

In one preferred embodiment, a  $\text{Fc}_\epsilon\text{R}$  molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a  $\text{Fc}_\epsilon\text{R}$  molecule (referred to herein as an anti- $\text{Fc}_\epsilon\text{R}$  antibody) or a compound that selectively binds to a detectable marker conjugated to a  $\text{Fc}_\epsilon\text{R}$  molecule.  $\text{Fc}_\epsilon\text{R}$  molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure® NeutrAvidin (available from Pierce, Rockford, IL).

In another preferred embodiment, a  $\text{Fc}_\epsilon\text{R}$  molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to herein as an anti-IgE reagent). Examples of such an anti-IgE reagent include, but are not limited to, a secondary antibody that is an anti-isotype antibody (e.g., an antibody that selectively binds to the constant region of an IgE), an antibody-binding bacterial surface protein (e.g., Protein A or Protein G), an antibody-binding cell (e.g., a B cell, a T cell, a natural killer cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an Fc receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9, CMI antibody #19, CMI antibody #59 and CMI antibody #71 (available from Custom Monoclonal International, West Sacramento, CA). In particular, as used herein, an anti-IgE antibody includes not only a complete antibody

but also any subunit or portion thereof that is capable of selectively binding to an IgE heavy chain constant region. For example, a portion of an anti-IgE reagent can include an Fab fragment or a F(ab')<sub>2</sub> fragment, which are described in detail in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc.,  
5 NY, 1996 (which is incorporated herein by this reference in its entirety).

In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to,  
10 plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic  
15 particle, and other particulates. A particularly preferred substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method to detect IgE is an immunosorbent assay. An  
20 immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgE in such a manner that the IgE is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of the present invention prior to exposure of the

capture molecule to a putative IgE-containing composition. An indicator molecule of the present invention detects the presence of an IgE bound to a capture molecule. As such, an indicator molecule preferably is not immobilized to the same substrate as a capture molecule prior to exposure of the capture molecule to a putative IgE-containing composition.

A preferred immunoabsorbent assay method includes a step of either: (a) binding an Fc<sub>ε</sub>R molecule to a substrate prior to contacting a Fc<sub>ε</sub>R molecule with a putative IgE-containing composition to form a Fc<sub>ε</sub>R molecule-coated substrate; or (b) binding a putative IgE-containing composition to a substrate prior to contacting a Fc<sub>ε</sub>R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a Fc<sub>ε</sub>R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

Both a capture molecule and an indicator molecule of the present invention are capable of binding to an IgE. Preferably, a capture molecule binds to a different region of an IgE than an indicator molecule, thereby allowing a capture molecule to be bound to an IgE at the same time as an indicator molecule. The use of a reagent as a capture molecule or an indicator molecule depends upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a Fc<sub>ε</sub>R molecule of the present invention is used as a capture molecule when the Fc<sub>ε</sub>R molecule is bound to a substrate. Alternatively, a Fc<sub>ε</sub>R molecule is used as an indicator molecule when the Fc<sub>ε</sub>R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or

indicator molecules include, but are not limited to, a Fc<sub>ε</sub>R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

An immunoabsorbent assay of the present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred secondary molecules of the present invention include, an antigen, an anti-IgE idiotypic antibody and an anti-IgE isotypic. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

In one embodiment, a desired antigen is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. Preferred antigens include those disclosed herein. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable (i.e., sufficient) to allow for antigen:IgE complex formation bound to the substrate (i.e., IgE in a sample binds to an antigen immobilized on a substrate). Excess non-bound material (i.e., material from the biological sample that has not bound to the antigen), if any, is removed from the substrate under conditions that retain antigen:IgE complex binding to the substrate. Preferred conditions are disclosed herein in the Examples section and generally in Sambrook et al., *ibid.* An indicator molecule that can selectively bind to an IgE bound to

the antigen, the indicator molecule can be conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family), is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the antigen:IgE complex. Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a  $Fc_\epsilon R$  molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

In one embodiment, a  $Fc_\epsilon R$  molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for  $Fc_\epsilon R$  molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain  $Fc_\epsilon R$  molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the  $Fc_\epsilon R$  is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the  $Fc_\epsilon R$  molecule:IgE complex. Preferably, the indicator molecule is conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family). Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an antigen that will bind to IgE in the biological sample or an anti-IgE isotype or idiotype antibody, either preferably being conjugated to fluorescein or biotin.



In one embodiment, an anti-IgE antibody (e.g., isotype or idiotype specific antibody) is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for anti-IgE antibody:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A  $\text{Fc}_\epsilon\text{R}$  molecule is added to the substrate and incubated to allow formation of a complex between the  $\text{Fc}_\epsilon\text{R}$  molecule and the anti-IgE antibody:IgE complex. Preferably, the  $\text{Fc}_\epsilon\text{R}$  molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess  $\text{Fc}_\epsilon\text{R}$  molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a biological sample collected from an animal is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for IgE binding to the substrate. Any IgE present in the bodily fluid is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A  $\text{Fc}_\epsilon\text{R}$  molecule is added to the substrate and incubated to allow formation of a complex between the  $\text{Fc}_\epsilon\text{R}$  molecule and the IgE. Preferably, the  $\text{Fc}_\epsilon\text{R}$  molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess  $\text{Fc}_\epsilon\text{R}$  molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

Another preferred method to detect IgE is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al.; each of these patent publications is incorporated by reference herein in its entirety. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to an antigen, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an IgE-binding composition. Preferred antigens include those disclosed herein. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF, carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds to IgE. A preferred labeling reagent is an antigen conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a  $Fc_\epsilon R$  molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilizing. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a  $Fc_\epsilon R$  molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an antigen, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone

located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes an absorbent located at the end of the flow path.

One embodiment of the present invention is an inhibition assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a  $\text{Fc}_\epsilon\text{R}$  molecule of the present invention and an isolated IgE known to bind to the  $\text{Fc}_\epsilon\text{R}$  molecule. The absence of binding of the  $\text{Fc}_\epsilon\text{R}$  molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

The present invention also includes kits to detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a human  $\text{Fc}_\epsilon$  receptor ( $\text{Fc}_\epsilon\text{R}$ ) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred  $\text{Fc}_\epsilon\text{R}$  molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the  $\text{Fc}_\epsilon\text{R}$  molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one or more antigens disclosed herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a  $\text{Fc}_\epsilon\text{R}$  molecule (e.g., avidin, streptavidin and ImmunoPure® NeutrAvidin when the detectable marker is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

A preferred embodiment of a kit of the present invention is a flea allergen kit comprising a flea allergen such as those disclosed herein. A particularly preferred flea allergen for use with a flea allergen kit includes a flea saliva product or a flea saliva protein.

Another preferred kit of the present invention is a general allergen kit comprising an allergen common to all regions of the United States and a human Fc $\epsilon$ R molecule of the present invention. As used herein, a “general allergen” kit refers to a kit comprising allergens that are found substantially throughout the United States (i.e., essentially not limited to certain regions of the United States). A general allergen kit provides an advantage over regional allergen kits because a single kit can be used to test an animal located in most geographical locations on the United States. Suitable and preferred general allergens for use with a general allergen kit of the present invention include those general allergens disclosed herein.

Another preferred kit of the present invention is a food allergen kit comprising a food allergen including beef, chicken, pork, a mixture of fish, such as cod, halibut or and tuna, egg, milk, Brewer’s yeast, whole wheat, corn, soybean, cheese and rice, and a human Fc $\epsilon$ R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

A preferred kit of the present invention includes those in which the allergen is immobilized to a substrate. If a kit comprises two or more antigens, the kit can comprise one or more compositions, each composition comprising one antigen. As such, each antigen can be tested separately. A kit can also contain two or more diagnostic reagents for IgE, additional isolated IgE antigens and/or antibodies as disclosed herein.

Particularly preferred are kits used in a lateral flow assay format. It is within the scope of the present invention that a lateral flow assay kit can include one or more lateral flow assay apparatuses. Multiple lateral flow apparatuses can be attached to each other at one end of each apparatus, thereby creating a fan-like structure.

In particular, a method and kit of the present invention are useful for diagnosing abnormal conditions in animals that are associated with changing levels of IgE.

Particularly preferred conditions to diagnose include allergies, parasitic infections and neoplasia. For example, a method and kit of the present invention are particularly useful  
5 for detecting flea allergy dermatitis (FAD), when such method or kit includes the use of a flea saliva antigen. FAD is defined as a hypersensitive response to fleabites. Preferably, a putative IgE-containing composition is obtained from an animal suspected of having FAD. Preferred animals include those disclosed herein, with dogs and cats being more preferred. In addition, methods and kits of the present invention are particularly useful  
10 for detecting helminth infection, in particular heartworm infection, when such methods or kits include the use of a helminth antigen, such as Di33. Preferably, a putative IgE-containing composition is obtained from an animal suspected of having a helminth infection. Preferred animals include those disclosed herein, with dogs and cats being more preferred.

15 The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

## Examples

### Example 1.

This example describes the construction of a recombinant baculovirus expressing a truncated portion of the  $\alpha$ -chain of the human  $\text{Fc}_\epsilon$  receptor.

Recombinant molecule pVL-nh $\text{Fc}_\epsilon\text{R}\alpha_{612}$ , containing a nucleic acid molecule encoding the extracellular domain of the  $\text{Fc}_\epsilon\text{R}$   $\alpha$  chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain ( $\alpha$  chain) of the human  $\text{Fc}_\epsilon$  receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, MA). The cDNA clone included an about 1198 nucleotide insert, referred to herein as nh $\text{Fc}_\epsilon\text{R}\alpha_{1198}$ . The nucleic acid sequence of the coding strand of nh $\text{Fc}_\epsilon\text{R}\alpha_{1198}$  is denoted herein as SEQ ID NO:1. Translation of SEQ ID NO:1 indicates that nucleic acid molecule nh $\text{Fc}_\epsilon\text{R}\alpha_{1198}$  encodes a full-length human  $\text{Fc}_\epsilon$  receptor  $\alpha$  chain protein of about 257 amino acids, referred to herein as Ph $\text{Fc}_\epsilon\text{R}\alpha_{257}$ , having amino acid sequence SEQ ID NO:2, assuming an open reading frame in which the initiation codon spans from about nucleotide 107 through about nucleotide 109 of SEQ ID NO:1 and the termination codon spans from about nucleotide 878 through about nucleotide 880 of SEQ ID NO:1. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e.,  $\text{Fc}_\epsilon\text{R}\alpha$  chain from which the signal sequence has been cleaved), denoted herein as Ph $\text{Fc}_\epsilon\text{R}\alpha_{232}$ , contains about 232 amino acids which is represented herein as SEQ ID NO:6. The nucleic acid molecule encoding Ph $\text{Fc}_\epsilon\text{R}\alpha_{232}$  is denoted herein as nh $\text{Fc}_\epsilon\text{R}\alpha_{696}$ , the coding strand of which is represented by SEQ ID NO:7.

To produce a secreted form of the extracellular domain of the Fc<sub>ε</sub>R α chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the Fc<sub>ε</sub>R α chain encoded by nhFc<sub>ε</sub>Rα<sub>1198</sub> were removed as follows. A Fc<sub>ε</sub>R α chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc<sub>ε</sub>Rα<sub>1198</sub> using a forward primer EJH 040 containing a *Bam*HI site, having the nucleic acid sequence 5' CGC GGA TCC TAT AAT ATG GCT CCT GCC ATG G 3' (denoted SEQ ID NO:8) and a reverse primer IgE ANTI-SENSE containing an *Eco*RI site, having the nucleic acid sequence 5' GGC GAA TTC TTA AGC TTT TAT TAC AG 3' (denoted herein as SEQ ID NO:9). The resulting PCR product was digested with *Bam*HI and *Eco*RI to produce nhFc<sub>ε</sub>Rα<sub>612</sub>. Nucleic acid molecule nhFc<sub>ε</sub>Rα<sub>612</sub> contained an about 591 nucleotide fragment encoding the extracellular domain of the human Fc<sub>ε</sub>R α chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as nucleic acid molecule nhFc<sub>ε</sub>Rα<sub>591</sub>, the coding strand of which has a nucleic acid sequence denoted SEQ ID NO:10. Translation of SEQ ID NO:10 indicates that nucleic acid molecule nhFc<sub>ε</sub>Rα<sub>612</sub> encodes a Fc<sub>ε</sub>R protein of about 197 amino acids, referred to herein as PhFc<sub>ε</sub>Rα<sub>197</sub>, having amino acid sequence SEQ ID NO:11. Nucleic acid molecule nhFc<sub>ε</sub>Rα<sub>612</sub> encodes a secretable form of the human Fc<sub>ε</sub>R α chain which does not possess a leader sequence, which is denoted herein as PhFc<sub>ε</sub>Rα<sub>172</sub> having amino acid sequence SEQ ID NO:13. The coding region for PhFc<sub>ε</sub>Rα<sub>172</sub> is denoted nhFc<sub>ε</sub>Rα<sub>516</sub>, the coding strand of which has a nucleic acid sequence denoted SEQ ID NO:12. The complement of SEQ ID NO:12 is represented herein by SEQ ID NO:14.

In order to produce a baculovirus recombinant molecule capable of directing the production of PhFc<sub>ε</sub>Rα<sub>197</sub>, the nucleic acid molecule nhFc<sub>ε</sub>Rα<sub>612</sub> was subcloned into unique



*Bam*HI and *Eco*RI sites of pVL1392 baculovirus shuttle plasmid (available from Pharmingen, San Diego, CA) to produce a recombinant molecule referred to herein as pVL-nhFc<sub>ε</sub>Rα<sub>612</sub>. The resultant recombinant molecule pVL-nhFc<sub>ε</sub>Rα<sub>612</sub> was verified for proper insert orientation by restriction mapping.

#### Example 2.

This example describes the production of PhFc<sub>ε</sub>Rα<sub>172</sub> protein.

The recombinant molecule pVL-nhFc<sub>ε</sub>Rα<sub>612</sub> was co-transfected with a linear Baculogold baculovirus DNA (available from Pharmingen) into *Trichoplusia ni* cells (available from Invitrogen Corp., San Diego, CA; High Five™ cells) using the following method. About 1.5 liter cultures of serum-free ex-Cell Medium (available from Invitrogen) were seeded with about 1 x 10<sup>6</sup> cells per ml of medium. The *Trichoplusia ni* cells were infected with recombinant molecule pVL-nhFc<sub>ε</sub>Rα<sub>612</sub> at a multiplicity of infection (MOI) of about 2 to about 5 particle forming units (pfu) per cell to produce recombinant cell *Trichoplusia ni*-pVL-nhFc<sub>ε</sub>Rα<sub>612</sub>. The infection was allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant protein PhFc<sub>ε</sub>Rα<sub>172</sub>. Following infection, cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

PhFc<sub>ε</sub>Rα<sub>172</sub> was purified from the culture medium described immediately above by affinity chromatography using an IgE antibody produced by the myeloma cell line U266DI (American Tissue Type Catalogue No. TIB196) linked to sepharose 4B. The amino acid composition and N-terminal amino acid sequence of the affinity purified PhFc<sub>ε</sub>Rα<sub>172</sub> were determined using methods standard in the art. The results indicated that PhFc<sub>ε</sub>Rα<sub>172</sub> was properly processed by the *Trichoplusia ni* cells.

### Example 3.

This example describes the biotinylation of a recombinant human Fc $\epsilon$ R alpha chain protein.

Affinity purified recombinant protein PhFc $\epsilon$ R $\alpha_{172}$ , prepared as described above in Example 2, was biotinylated as follows. About 440 micrograms ( $\mu$ g) of PhFc $\epsilon$ R $\alpha_{172}$  were diluted in about 1.5 milliliter (ml) of acetate buffer (0.1 M NaAc, pH 5.5) containing about 200 microliter ( $\mu$ l) of 0.1 M NaIO $_4$ . The mixture was incubated for about 20 minutes, on ice, and about 2  $\mu$ l of glycerol was added following the incubation. The mixture was then dialyzed against about 2 liters of acetate buffer in a 3 ml Slide-A-Lyzer cassette (available from Pierce, Rockford, IL), 2 times for about 2 hours each time. About 3.72  $\mu$ g of biotin-LC-hydrazide (available from Pierce) was dissolved in about 200  $\mu$ l of dimethylsulfoxide (DMSO) and injected into the cassette. The cassette was then rocked at room temperature for about 2 hours. Following the incubation, the mixture containing recombinant protein and biotin dialyzed 3 times, a first time for about 18 hours and two times for about 2 hours, each time at 5 $^{\circ}$ C against phosphate buffered saline. The biotinylated protein was recovered from the dialysis, and is referred to herein as PhFc $\epsilon$ R $\alpha_{172}$ -BIOT.

### Example 4.

This example describes detection of canine IgE in a solid-phase ELISA using PhFc $\epsilon$ R $\alpha_{172}$ -BIOT.

Wells of two Immulon II microtiter plates (available from Dynatech, Alexandria, VA) were coated with duplicate samples of about 100  $\mu$ l/well of various concentrations of purified canine IgE as denoted in Fig. 1. The canine IgE was obtained from a canine IgE producing

hybridoma, such as heterohybridoma 2.39 (described in Gebhard et al., *Immunology* 85:429-434, 1995) and was diluted in a CBC buffer (15 mM Na<sub>2</sub>CO<sub>3</sub> and 34.8 mM NaHCO<sub>3</sub>, pH 9.6. The coated plates were incubated overnight at 4°C. Following incubation, the canine IgE-containing solution was removed from each plate, and the plates were blotted dry. The plates were then blocked using about 200 µl/well of 0.25% bovine serum albumin (BSA) contained in phosphate buffered saline (PBSB) for about 1 hour at room temperature. The plates were then washed four times with 0.05% Tween-20 in PBS (PBST) using an automatic washer (available from Dynatech). Experimental samples consisting of about 100 µl/well of a 1:4000 dilution of 40 µg/ml PhFc<sub>ε</sub>Rα<sub>172</sub>-BIOT (about 145 µg/ml; described in Example 3), contained in PBSB with 0.05% Tween-20 (PBSBT) were added to each well of one plate coated with canine IgE. Control samples consisting of about 100 µl of biotinylated anti-canine IgE monoclonal antibody D9 (supplied by Dr. DeBoer, U. of Wisconsin, Madison, WI) were added to each well of the other plate coated with canine IgE. The plates were incubated for 1 hour at room temperature and then washed four times with PBST. About 100 µl of about 0.25 ug/ml streptavidin conjugated to horseradish peroxidase (available from Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD; diluted in PBST) was added to each well that received experimental or control samples. The plates were then incubated for 1 hour at room temperature and washed four times with PBST. About 100 µl of TMB substrate (available from available from KPL), that had been pre-warmed to room temperature, was added. Plates were then incubated for 10 minutes at room temperature and then about 100 µl/well of Stop Solution (available from KPL) was added. Optical densities of wells were read on a Spectramax Microtiter Plate (available from Molecular Devices Inc.) reader at 450 nm within 10 minutes of adding the stop solution.

The results shown in Fig. 1 indicate that the alpha chain of human  $\text{Fc}_\epsilon\text{R}$  detects the presence of canine IgE (closed circles) in a solid-phase assay in a similar manner as the control antibody that binds specifically to canine IgE (D9; open circles).

#### Example 5.

This example describes detection of plant allergen-specific canine IgE using  $\text{PhFc}_\epsilon\text{R}\alpha_{172}$ -BIOT.

Multiple wells of an Immulon II microtiter plate (available from Dynatech) were coated with either about 100  $\mu\text{l}$ /well of 1  $\mu\text{g}/\text{ml}$  of Kentucky Blue Grass allergen or about 100  $\mu\text{l}$ /well of about 1  $\mu\text{g}/\text{ml}$  of Green Ash allergen (both available from Greer Inc., Lenoir, NC) both diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Two different pools of canine sera were then added to the antigen-coated wells. The first pool consisted of sera isolated from 8 dogs reported to be allergen reactive. The second pool consisted of sera isolated from 8 dogs reported to be allergen non-reactive. Each pool of sera was diluted 1:10 or 1:100 in PBST. About 100  $\mu\text{l}$  of each concentration of each diluted sera sample was added to the wells and incubated for 1 hour at room temperature. The plate was then washed four times with PBST. About 100  $\mu\text{l}$ /well of a 1:4000 dilution of 40  $\mu\text{g}/\text{ml}$   $\text{PhFc}_\epsilon\text{R}\alpha_{172}$ -BIOT (described in Example 3), contained in PBSBT was added to the antigen-coated wells. The plate was incubated for 1 hour at room temperature. The plate was then washed four times with PBST. About 100  $\mu\text{l}$ /well of about 0.25  $\mu\text{g}/\text{ml}$  of neutravidin conjugated to horseradish peroxidase (available from Pierce) contained in PBSBT, was added. The plate was incubated for 1 hour at room temperature. The plate was then washed

and the presence of neutravidin bound to the plate detected using the method described in Example 4.

The results shown in Fig. 2 indicate that the alpha chain of human Fc $\epsilon$ R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common tree allergen. In addition, detection of canine IgE antibodies is dose dependent.

#### Example 6.

This example describes detection of total canine IgE using PhFc $\epsilon$ R $\alpha_{172}$ -BIOT.

Multiple wells of an Immulon II microtiter plate (available from Dynatech) were coated with about 100  $\mu$ l/well of about 1  $\mu$ g/ml CMI anti-canine IgE antibody #6 (available from Custom Monoclonals International, West Sacramento, CA) diluted in CBC buffer. The plate was incubated overnight at 4 $^{\circ}$ C. The plate was blocked and washed as described in Example 4. About 100  $\mu$ l/well of a 1:60 dilution in PBSBT of sera samples from a variety of sources were then added to multiple wells coated with anti-IgE antibody. The samples included: (1) serum from a dog known to be allergic to flea saliva; (2) serum from dogs infected with *D. immitis*; (3) and (4) a pool of dog sera from defined as canine allergy calibrators (available from BioProducts DVM, Tempe, AZ); (5) pools of dog sera containing antibodies that have low binding to Kentucky Blue Grass allergen; (6) pools of dog sera that have high binding to Kentucky Blue Grass allergen; (7) a pool of dog sera from dogs known to be allergic to flea saliva, the sample was heat inactivated (at 56 $^{\circ}$ C for 4 hours); (8) a pool of dog sera from dogs known to be allergic to flea saliva; or (9) a pool of dog sera from dogs raised in a barrier facility (i.e., negative control). A set of positive control samples consisting of IgE derived from the canine heterohybridoma described in Example 4 were also added to the plate to generate a standard

curve. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. The presence of canine IgE was detected using either about 100  $\mu$ l/well of a 1:4000 dilution of 40  $\mu$ g/ml PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT (described in Example 3) or about 100  $\mu$ l/well of about 1  $\mu$ g/ml CMI anti-canine IgE antibody #19 (available from Custom Monoclonals International), both contained in PBSBT. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25  $\mu$ g/ml streptavidin conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4. The optical density readings obtained for the control samples were used to generate a standard curve that was used to determine the total IgE bound to wells that had received test samples.

The results shown in Fig. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of human Fc $_{\epsilon}$ R in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts of IgE in the heat treated sample (Sample 7) indicates that the antibody detected by PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT is IgE. In addition, the results indicate that PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT is an effective reagent for detecting IgE that binds to allergen Kentucky Blue Grass, Samples 5 and 6), as well as a parasite antigen (*D. Immitis*, Sample 2).

#### Example 7.

This example describes detection of canine IgE in dog sera isolated from dogs known to be allergic to flea saliva, using PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100  $\mu$ l/well of varying concentrations of flea saliva recombinant protein fspN (described in PCT Patent

Publication No. WO 96/11271, *ibid.*; concentrations shown in Fig. 4) diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was then blocked and washed as described in Example 4. About 100 µl/well of a 1:10 dilution in PBSBT of a pool of sera isolated from dogs known to produce IgE that binds specifically to flea saliva. Some wells did not receive dog sera so that background binding levels could be determined. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100 µl/well of a 1:4000 dilution of 40 µg/ml PhFc<sub>ε</sub>Rα<sub>172</sub>-BIOT (described in Example 3) contained in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25 ug/ml streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of human Fc<sub>ε</sub>R.

#### Example 8.

This example describes detection of total canine IgE in dog sera isolated from dogs known to be allergic to flea saliva, heartworm-infected dogs and specific pathogen free (SPF) dogs, using PhFc<sub>ε</sub>Rα<sub>172</sub>-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100 µl/well of about 1 µg/ml CMI anti-canine IgE antibody #6 (available from Custom Monoclonals International) in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. About 100 µl/well of different samples of IgE-containing fluids in PBSBT were added to multiple wells coated with the anti-canine IgE

antibody. The samples included: (1) 100  $\mu\text{g/ml}$  of canine IgE purified from the heterohybridoma described in Example 4; (2) a 1:10 dilution of a pool of sera from dogs known to be allergic to flea saliva, (3) a 1:10 dilution of the same sera pool as in (2) but heat inactivated; (4) a 1:10 dilution of serum from a dog known to have clinical flea allergy dermatitis (dog CPO2); (5) a 1:10 dilution of heat inactivated CPO2 serum; (6) a 1:10 dilution of serum from a heartworm-infected dog (dog 417); (7) a 1:10 dilution of heat inactivated 417 serum; (8) a 1:10 dilution of a pool of sera from heartworm-infected dogs; (9) a 1:10 dilution of the same sera pool as in (8) but heat inactivated; and (10) a pool of sera from dogs raised in a barrier facility. Each sample was diluted in PBSBT. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100  $\mu\text{l/well}$  of a 1:4000 dilution of 40  $\mu\text{g/ml}$  PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25  $\mu\text{g/ml}$  streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 5 indicate that canine IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of human Fc $_{\epsilon}$ R. In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by Fc $_{\epsilon}$ R alpha chain is an epsilon isotype antibody and not another isotype.

#### Example 9.

This example describes detection of IgE that specifically binds to flea saliva, using PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT.



Multiple wells of an Immulon II microtiter plate were coated with about 100  $\mu$ l/well of about 0.1  $\mu$ g/ml of flea saliva collected using the method described in PCT Patent Publication No. WO 96/11271, *ibid.*, in CBC buffer. The plate was incubated, blocked and washed as described in Example 4. The IgE-containing samples described in Example 8 were then applied to the flea saliva coated plate. The plate was then treated using the method described in Example 8.

The results shown in Fig. 6 indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of human Fc $\epsilon$ R. In addition, the absence of colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by Fc $\epsilon$ R alpha chain is an epsilon isotype antibody.

#### Example 10.

This example describes the detection of feline IgE using PhFc $\epsilon$ R $\alpha_{172}$ -BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100  $\mu$ l/well of about 10  $\mu$ g/ml Di33 protein (described in U.S. Patent Application Serial No. 08/715,628, *ibid.*) or 10  $\mu$ g/ml crude homogenate of heartworm, both in CBC buffer. Crude homogenate of heartworm is the clarified supernatant of adult heartworms homogenized in PBS. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Serum samples from 2 heartworm infected cats were then added to Di33-coated wells and to heartworm antigen-coated wells. About 100  $\mu$ l/well of a 1:10 dilution in PBSBT of sera from heartworm-infected cat # AXH3 or from cat #MGC2 were added to the plate. Negative control samples consisting of serum from pre-infection bleeds of cat #AXH3 and cat# MGC2 were also added to the plate at a dilution of 1:10 in PBSBT. A positive control sample consisting of a pool

of sera from heartworm-infected dogs was also added to the plate at a dilution of 1:10 in PBSBT. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100  $\mu$ l/well of a 1:4000 dilution of 40  $\mu$ g/ml PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with 1:4000 dilution of a 0.5 mg/ml solution of streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 7 indicate that feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of human Fc $_{\epsilon}$ R.

#### Example 11.

This example describes detection of feline IgE using PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT.

Multiple wells of an Immulon II microtiter plate were coated with Di33 as described in Example 10, in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Serum samples from 2 heartworm infected cats were then added to Di33-coated wells. About 100  $\mu$ l/well of a 1:10 dilution in PBSBT of serum from heartworm-infected cat # MGC2 and a pool of sera from heartworm-infected cats, as well as heat inactivated samples of each of these sera, were added to the plate. A positive control sample consisting of a pool of sera from heartworm-infected dogs was also added to the plate at a dilution of 1:10 in PBSBT. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100  $\mu$ l/well of a 1:4000 dilution of 40  $\mu$ g/ml PhFc $_{\epsilon}$ R $\alpha_{172}$ -BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with streptavidin-conjugated to

horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 8 indicate that feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of human  $Fc_\epsilon R$ . In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by  $Fc_\epsilon R$  alpha chain is an epsilon isotype antibody.

#### Example 12

This example describes detection of equine IgE in a solid-phase ELISA using  $PhFc_\epsilon R\alpha_{172}$ -BIOT.

Horse sera from a horse known to be allergic to certain allergens and horse sera from a horse known not to be allergic the same allergens, were assayed for the presence of IgE using  $PhFc_\epsilon R\alpha_{172}$ -BIOT as follows. A North Atlantic/Ohio Valley Regional Panel plate of a Canitec™ Allergen-Specific IgE Kit (available from BioProducts DVM) was blocked and washed as described in Example 4. Two samples of about 1:10 dilutions of the two horse sera were prepared using PBSBT. The two samples were added to the blocked plate and the plate was incubated for 1 hour at room temperature. The plate was washed as described in Example 4. About 100  $\mu$ l/well of a 1:4000 dilution of 40  $\mu$ g/ml  $PhFc_\epsilon R\alpha_{172}$ -BIOT (described in Example 3), contained in PBSBT was added to each well. The plate was then washed, contacted with 1:4000 dilution of a 0.5 mg/ml solution of streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 9 indicate that equine IgE from a horse known to be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of human Fc<sub>ε</sub>R.

#### Example 13

This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with human Fc<sub>ε</sub>R alpha chain.

Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a human Fc<sub>ε</sub>R alpha chain (referred to herein as RBL-hFc<sub>ε</sub>R cells; described in Miller et al., *Science* 244:334-337, 1989) were used to detect canine IgE as follows. About 4 x 10<sup>4</sup> RBL-hFc<sub>ε</sub>R cells contained in Earles Modified Eagles Medium containing 10% fetal bovine serum (EMEM-FBS) were added to each well of 96-well flat bottom tissue culture plates. The RBL-hFc<sub>ε</sub>R cells were incubated overnight at 37°C. Following the incubation the plates were washed 4 times with PBST. The cells were then fixed for about 2 minutes using about 200 µl per well of absolute alcohol at room temperature. The plates were then washed 8 times with PBST to remove residual alcohol.

Serial dilutions in EMEM-FBS (concentrations shown in Fig. 10) were prepared using a pool of sera from dogs infected with heartworm. Serial dilutions in EMEM-FBS (concentrations shown in Fig. 11) were prepared using a pool of sera from dogs sensitized to flea saliva. Additional samples were prepared in which both pools of sera were heat inactivated for about 4 hours at 56°C. The heat treated samples were diluted as described above.

About 100 µl of each dilution of each serum sample was added to separate wells containing fixed RBL-hFc<sub>ε</sub>R cells and the plates were incubated at 37°C for about 1 hour.

Following the incubation, the plates were washed 4 times with PBST. About 5 µg of a murine IgG monoclonal antibody anti-canine IgE antibody (i.e., Custom Monoclonal Antibody #71; available from Custom Monoclonal International) in 100 µl of EMEM-FBS was added to each well. The plates were incubated for about 30 minutes at 37°C. Following the incubation, the plates were washed 4 times with PBST. About 100 ng of horseradish peroxidase labelled donkey anti-murine IgG (available from Jackson Laboratories, Westgrove, PA) in 100 µl of EMEM-FBS was added to each well, and the plates were incubated for about 30 minutes at room temperature. Following the incubation, the plates were washed 4 times with PBST. The presence of anti-murine IgG bound to the plates thereby indicating the ability of RBL-hFc<sub>ε</sub>R cells to bind to canine IgE was detected using the method described in Example 4.

The results shown in Fig. 10 indicate that canine IgE from heartworm-infected dogs (◆) is detected using RBL-h Fc<sub>ε</sub>R cells expressing the alpha chain of human Fc<sub>ε</sub>R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (■) indicates that antibody detected by the Fc<sub>ε</sub>R alpha chain on the RBL-h Fc<sub>ε</sub>R cells is an epsilon isotype antibody. Similarly, the results shown in Fig. 11 indicate that canine IgE from dogs sensitized with flea saliva (◆) is detected using RBL-h Fc<sub>ε</sub>R cells expressing the alpha chain of human Fc<sub>ε</sub>R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (■) indicates that antibody detected by the Fc<sub>ε</sub>R alpha chain on the RBL-h Fc<sub>ε</sub>R cells is an epsilon isotype antibody.

SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR § 1.821. A copy in computer readable form is also submitted herewith.

5 Applicants assert pursuant to 37 CFR § 1.82(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:13 submitted herewith are the same.

(1) GENERAL INFORMATION:

- 10 (i) APPLICANT: Frank, Glenn R.  
Porter, James P.  
Rushlow, Keith E.  
Wassom, Donald L.
- (ii) TITLE OF INVENTION: Method to Detect IgE
- (iii) NUMBER OF SEQUENCES: 13
- 15 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Carol Talkington Verser, Ph.D.  
Heska Corporation  
(B) STREET: 1825 Sharp Point Drive  
20 (C) CITY: Fort Collins  
(D) STATE: Colorado  
(E) COUNTRY: USA  
(F) ZIP: 80525
- 25 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: Windows 95  
(D) SOFTWARE: WordPerfect for Windows, Version 7.0
- 30 (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: 08/756,387  
(B) FILING DATE: November 26, 1996  
(C) CLASSIFICATION:
- 35 (vii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Verser, Carol Talkington  
(B) REGISTRATION NUMBER: 37,459  
(C) REFERENCE/DOCKET NUMBER: DI-1
- (viii) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 970/493-7272  
(B) TELEFAX: 970/484-9505

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1198 nucleotides  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 107..877

(iv) SEQUENCE DESCRIPTION: SEQ ID NO:1

	TACTAAGAGT CTCCAGCATC CTCCACCTGT CTACCACCGA GCATGGGCCT ATATTTGAAG	60
	CCTTAGATCT CTCCAGCACA GTAAGCACCA GGAGTCCATG AAGAAG ATG GCT CCT	115
		Met Ala Pro
		1
	GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG TTC TTC GCT	163
	Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu Phe Phe Ala	
		5 10 15
	CCA GAT GGC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC	211
	Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val Ser Leu Asn	
		20 25 30 35
	CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT CTT ACA TGT	259
	Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys	
		40 45 50
	AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG TTC CAC AAT	307
	Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn	
		55 60 65
	GGC AGC CTT TCA GAA GAG ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC	355
	Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala	
		70 75 80
	AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA CAA GTT AAT	403
	Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn	
		85 90 95
	GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GAC TGG CTG CTC CTT	451
	Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu	
		100 105 110 115
	CAG GCC TCT GCT GAG GTG GTG ATG GAG GGC CAG CCC CTC TTC CTC AGG	499
	Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg	
		120 125 130
	TGC CAT GGT TGG AGG AAC TGG GAT GTG TAC AAG GTG ATC TAT TAT AAG	547
	Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys	
		135 140 145
	GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC AAC ATC TCC ATT	595
	Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile	
		150 155 160

	ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT ACG GGC AAA	643
	Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Cys Thr Gly Lys	
	165 170 175	
5	GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA	691
	Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile	
	180 185 190 195	
	AAA GCT CCG CGT GAG AAG TAC TGG CTA CAA TTT TTT ATC CCA TTG TTG	739
	Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu	
	200 205 210	
10	GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA TTA TTT ATC TCA ACT CAG	787
	Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile Ser Thr Gln	
	215 220 225	
	CAG CAG GTC ACA TTT CTC TTG AAG ATT AAG AGA ACC AGG AAA GGC TTC	835
15	Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe	
	230 235 240	
	AGA CTT CTG AAC CCA CAT CCT AAG CCA AAC CCC AAA AAC AAC TGA	880
	Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn Asn	
	245 250 255	
	TATAATTACT CAAGAAATAT TTGCAACATT AGTTTTTTTC CAGCATCAGC AATTGCTACT	940
20	CAATTGTCAA ACACAGCTTG CAATATACAT AGAAACGTCT GTGCTCAAGG ATTTATAGAA	1000
	ATGCTTCATT AAAGTGAAGT AAAGTGGTTA AGTGGCATGT AATAGTAAGT GCTCAATTAA	1060
	CATTGGTTGA ATAAATGAGA GAATGAATAG ATTCATTTAT TAGCATTGT AAAAGAGATG	1120
	TTCAATTTCA ATAAAATAAA TATAAAACCA TGTAACAGAA TGCTTCTGAG TAAAAA	1180
	AAAAA	1198
25	(2) INFORMATION FOR SEQ ID NO:2:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 257 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
30	(ii) MOLECULE TYPE: protein	
	(iii) SEQUENCE DESCRIPTION: SEQ ID NO:2:	
	Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu	
	1 5 10 15	
35	Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val	
	20 25 30	
	Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr	
	35 40 45	
	Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp	
	50 55 60	
40	Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile	
	65 70 75 80	



Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln  
85 90 95

Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp  
100 105 110

5 Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu  
115 120 125

Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile  
130 135 140

10 Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn  
145 150 155 160

Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys  
165 170 175

Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile  
180 185 190

15 Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile  
195 200 205

Pro Leu Leu Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile  
210 215 220

20 Ser Thr Gln Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg  
225 230 235 240

Lys Gly Phe Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn  
245 250 255

Asn

## (2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1198 nucleotides  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTTTTTTT TTTTTTTTTT TTTTTTTACT CAGAAGCATT CTGTTACATG GTTTTATATT 60  
TATTTTATTG AAATTGAACA TCTCTTTTAC AAATGCTAAT AAATGAATCT ATTCATTCTC 120  
TCATTTATTC AACCAATGTT AATTGAGCAC TTACTATTAC ATGCCACTTA ACCAGTTTCA 180  
35 CTCAGTTTAA TGAAGCATTT CTATAAATCC TTGAGCACAG ACGTTTCTAT GTATATTGCA 240  
AGCTGTGTTT GACAATTGAG TAGCAATTGC TGATGCTGGA AAAAACTAA TGTTGCAAAT 300  
ATTTCTTGAG TAATTATATC AGTTGTTTTT GGGGTTTGGC TTAGGATGTG GGTTGAGAAG 360  
TCTGAAGCCT TTCCTGGTTC TCTTAATCTT CAAGAGAAAT GTGACCTGCT GCTGAGTTGA 420  
GATAAATAAT CCTGTGTCCA CAGCAAACAG AATCACCACC AACAAATGGGA TAAAAAATTG 480

TAGCCAGTAC TTCTCACGCG GAGCTTTTAT TACAGTAATG TTGAGGGGCT CAGACTCATA 540  
 GTCCAGCTGC CACACTTTGC CCGTACAGTA GTAGGTTCCA CTGTCTTCAA CTGTGGCATT 600  
 TGTAATGGAG ATGTTGTGGT TCTCATACCA GTACTTGAGA GCTTCACCAT CCTTATAATA 660  
 GATCACCTTG TACACATCCC AGTTCCTCCA ACCATGGCAC CTGAGGAAGA GGGGCTGGCC 720  
 5 CTCCATCACC ACCTCAGCAG AGGCCTGAAG GAGCAGCCAG TCACTGAAGA CTTCCAGGTA 780  
 CACAGGTTCA CTCTCATTAA CTTGTTGGTG CTGACATTTG TATTCTCCAC TGTCTTCAAA 840  
 TTTGGCATTG ACAATATTCA AACTTGAATT TGTCTCTTCT GAAAGGCTGC CATTGTGGAA 900  
 CCATTTGGTG GAACTGACTT CAAAGAAATT GTTCCCATTA CATGTAAGAG TCACATTCTC 960  
 TCCTTTAAAT ATTCTATTCC ATGGAGGGTT CAAGGAGACC TTAGGTTTCT GAGGGACTGC 1020  
 10 TAACACGCCA TCTGGAGCGA AGAACAGTAA GGCTACACAC AGTAGAGTAG GGGATTCCAT 1080  
 GGCAGGAGCC ATCTTCTTCA TGGACTCCTG GTGCTTACTG TGCTGGAGAG ATCTAAGGCT 1140  
 TCAATATAG GCCCATGCTC GGTGGTAGAC AGGTGGAGGA TGCTGGAGAC TCTTAGTA 1198

## (2) INFORMATION FOR SEQ ID NO:4:

- 15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 774 nucleotides  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: cDNA  
 20 (iii) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..774  
 (iv) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 ATG GCT CCT GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG 48  
 Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu  
 1 5 10 15  
 TTC TTC GCT CCA GAT GGC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC 96  
 Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val  
 20 25 30  
 30 TCC TTG AAC CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT 144  
 Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr  
 35 40 45  
 CTT ACA TGT AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG 192  
 Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp  
 50 55 60  
 TTC CAC AAT GGC AGC CTT TCA GAA GAG ACA AAT TCA AGT TTG AAT ATT 240  
 Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile  
 65 70 75 80  
 40 GTG AAT GCC AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA 288  
 Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln  
 85 90 95

	CAA GTT AAT GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GAC TGG	336
	Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp	
	100 105 110	
5	CTG CTC CTT CAG GCC TCT GCT GAG GTG GTG ATG GAG GGC CAG CCC CTC	384
	Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu	
	115 120 125	
	TTC CTC AGG TGC CAT GGT TGG AGG AAC TGG GAT GTG TAC AAG GTG ATC	432
	Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile	
	130 135 140	
10	TAT TAT AAG GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC AAC	480
	Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn	
	145 150 155 160	
	ATC TCC ATT ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT	528
15	Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys	
	165 170 175	
	ACG GGC AAA GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT	576
	Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile	
	180 185 190	
20	ACT GTA ATA AAA GCT CCG CGT GAG AAG TAC TGG CTA CAA TTT TTT ATC	624
	Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile	
	195 200 205	
	CCA TTG TTG GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA TTA TTT ATC	672
	Pro Leu Leu Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile	
	210 215 220	
25	TCA ACT CAG CAG CAG GTC ACA TTT CTC TTG AAG ATT AAG AGA ACC AGG	720
	Ser Thr Gln Gln Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg	
	225 230 235 240	
	AAA GGC TTC AGA CTT CTG AAC CCA CAT CCT AAG CCA AAC CCC AAA AAC	768
30	Lys Gly Phe Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn	
	245 250 255	
	AAC TGA	774
	Asn	

## (2) INFORMATION FOR SEQ ID NO:5:

35	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 774 nucleotides	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
40	(iii) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
	TCAGTTGTTT TTGGGGTTTG GCTTAGGATG TGGGTTTCAGA AGTCTGAAGC CTTTCCTGGT	60
	TCTCTTAATC TTCAAGAGAA ATGTGACCTG CTGCTGAGTT GAGATAAATA ATCCTGTGTC	120
	CACAGCAAAC AGAATCACCA CCAACAATGG GATAAAAAAT TGTAGCCAGT ACTTCTCACG	180
	CGGAGCTTTT ATTACAGTAA TGTTGAGGGG CTCAGACTCA TAGTCCAGCT GCCACACTTT	240

GCCCGTACAG TAGTAGGTTT CACTGTCTTC AACTGTGGCA TTTGTAATGG AGATGTTGTG 300  
 GTTCTCATAC CAGTACTTGA GAGCTTCACC ATCCTTATAA TAGATCACCT TGTACACATC 360  
 CCAGTTCCTC CAACCATGGC ACCTGAGGAA GAGGGGCTGG CCTCCATCA CCACCTCAGC 420  
 AGAGGCCTGA AGGAGCAGCC AGTCACTGAA GACTTCCAGG TACACAGGTT CACTCTCATT 480  
 5 AACTTGTTGG TGCTGACATT TGTATTCTCC ACTGTCTTCA AATTTGGCAT TCACAATATT 540  
 CAAACTTGAA TTTGTCTCTT CTGAAAGGCT GCCATTGTGG AACCATTTGG TGGAACGAC 600  
 TTCAAAGAAA TTGTTCCCAT TACATGTAAG AGTCACATTC TCTCCTTTAA ATATTCTATT 660  
 CCATGGAGGG TTCAAGGAGA CCTTAGGTTT CTGAGGGACT GCTAACACGC CATCTGGAGC 720  
 GAAGAACAGT AAGGCTACAC ACAGTAGAGT AGGGGATTCC ATGGCAGGAG CCAT 774

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 232 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile  
 1 5 10 15  
 Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe  
 20 20 25 30  
 Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu  
 35 40 45  
 Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly  
 50 55 60  
 25 Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr  
 65 70 75 80  
 Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val  
 85 90 95  
 30 Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn  
 100 105 110  
 Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys  
 115 120 125  
 Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu  
 130 135 140  
 35 Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr  
 145 150 155 160  
 Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg Glu Lys  
 165 170 175

Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala  
 180 185 190  
 Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu  
 195 200 205  
 5 Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His  
 210 215 220  
 Pro Lys Pro Asn Pro Lys Asn Asn  
 225 230

## (2) INFORMATION FOR SEQ ID NO:7:

10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 699 nucleotides  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(iii) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..699

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:7:

20 GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC CCT CCA TGG AAT AGA ATA 48  
 Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile  
 1 5 10 15

25 TTT AAA GGA GAG AAT GTG ACT CTT ACA TGT AAT GGG AAC AAT TTC TTT 96  
 Lys Gly Glu Asn Val Thr Leu Thr Cys Phe Asn Gly Asn Asn Phe Phe  
 20 25 30

GAA GTC AGT TCC ACC AAA TGG TTC CAC AAT GGC AGC CTT TCA GAA GAG 144  
 Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu  
 35 40 45

30 ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC AAA TTT GAA GAC AGT GGA 192  
 Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly  
 50 55 60

GAA TAC AAA TGT CAG CAC CAA CAA GTT AAT GAG AGT GAA CCT GTG TAC 240  
 Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr  
 65 70 75 80

35 CTG GAA GTC TTC AGT GAC TGG CTG CTC CTT CAG GCC TCT GCT GAG GTG 288  
 Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val  
 85 90 95

40 GTG ATG GAG GGC CAG CCC CTC TTC CTC AGG TGC CAT GGT TGG AGG AAC 336  
 Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn  
 100 105 110

TGG GAT GTG TAC AAG GTG ATC TAT TAT AAG GAT GGT GAA GCT CTC AAG 384  
 Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys  
 115 120 125

45 TAC TGG TAT GAG AAC CAC AAC ATC TCC ATT ACA AAT GCC ACA GTT GAA 432  
 Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu  
 130 135 140

	GAC AGT GGA ACC TAC TAC TGT ACG GGC AAA GTG TGG CAG CTG GAC TAT	480
	Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr	
	145 150 155 160	
5	GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA AAA GCT CCG CGT GAG AAG	528
	Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg Glu Lys	
	165 170 175	
	TAC TGG CTA CAA TTT TTT ATC CCA TTG TTG GTG GTG ATT CTG TTT GCT	576
	Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala	
	180 185 190	
10	GTG GAC ACA GGA TTA TTT ATC TCA ACT CAG CAG CAG GTC ACA TTT CTC	624
	Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu	
	195 200 205	
	TTG AAG ATT AAG AGA ACC AGG AAA GGC TTC AGA CTT CTG AAC CCA CAT	672
	Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His	
15	210 215 220	
	CCT AAG CCA AAC CCC AAA AAC AAC TGA	699
	Pro Lys Pro Asn Pro Lys Asn Asn	
	225 230	

## (2) INFORMATION FOR SEQ ID NO:8:

- 20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: primer
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGGATCCT ATAAATATGG CTCCTGCCAT GG 32

## (2) INFORMATION FOR SEQ ID NO:9:

- 30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 26 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: primer
- 35 (iii) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGCGAATTCT TAAGCTTTTA TTACAG 26

## (2) INFORMATION FOR SEQ ID NO:10:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 591 nucleotides  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

## (iii) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..591

## (iv) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5	ATG GCT CCT GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG	48
	Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu	
	1 5 10 15	
10	TTC TTC GCT CCA GAT GGC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC	96
	Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val	
	20 25 30	
15	TCC TTG AAC CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT	144
	Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr	
	35 40 45	
20	CTT ACA TGT AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG	192
	Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp	
	50 55 60	
25	TTC CAC AAT GGC AGC CTT TCA GAA GAG ACA AAT TCA AGT TTG AAT ATT	240
	Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile	
	65 70 75 80	
30	GTG AAT GCC AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA	288
	Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln	
	85 90 95	
35	CAA GTT AAT GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GAC TGG	336
	Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp	
	100 105 110	
40	CTG CTC CTT CAG GCC TCT GCT GAG GTG GTG ATG GAG GGC CAG CCC CTC	384
	Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu	
	115 120 125	
45	TTC CTC AGG TGC CAT GGT TGG AGG AAC TGG GAT GTG TAC AAG GTG ATC	432
	Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile	
	130 135 140	
50	TAT TAT AAG GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC AAC	480
	Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn	
	145 150 155 160	
55	ATC TCC ATT ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT	528
	Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys	
	165 170 175	
60	ACG GGC AAA GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT	576
	Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile	
	180 185 190	
65	ACT GTA ATA AAA GCT	591
	Thr Val Ile Lys Ala	
	195	

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 197 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu  
 1 5 10 15  
 Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val  
 20 25 30  
 Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr  
 35 40 45  
 Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp  
 50 55 60  
 Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile  
 65 70 75 80  
 Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln  
 85 90 95  
 Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp  
 100 105 110  
 Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu  
 115 120 125  
 Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile  
 130 135 140  
 Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn  
 145 150 155 160  
 Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys  
 165 170 175  
 Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile  
 180 185 190  
 Thr Val Ile Lys Ala  
 195

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 nucleotides  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..516



## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

	GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC CCT CCA TGG AAT AGA ATA	48
	Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile	
	1 5 10 15	
5	TTT AAA GGA GAG AAT GTG ACT CTT ACA TGT AAT GGG AAC AAT TTC TTT	96
	Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe	
	20 25 30	
10	GAA GTC AGT TCC ACC AAA TGG TTC CAC AAT GGC AGC CTT TCA GAA GAG	144
	Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu	
	35 40 45	
	ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC AAA TTT GAA GAC AGT GGA	192
	Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly	
	50 55 60	
15	GAA TAC AAA TGT CAG CAC CAA CAA GTT AAT GAG AGT GAA CCT GTG TAC	240
	Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr	
	65 70 75 80	
	CTG GAA GTC TTC AGT GAC TGG CTG CTC CTT CAG GCC TCT GCT GAG GTG	288
	Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val	
	85 90 95	
20	GTG ATG GAG GGC CAG CCC CTC TTC CTC AGG TGC CAT GGT TGG AGG AAC	336
	Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn	
	100 105 110	
25	TGG GAT GTG TAC AAG GTG ATC TAT TAT AAG GAT GGT GAA GCT CTC AAG	384
	Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys	
	115 120 125	
	TAC TGG TAT GAG AAC CAC AAC ATC TCC ATT ACA AAT GCC ACA GTT GAA	432
	Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu	
	130 135 140	
30	GAC AGT GGA ACC TAC TAC TGT ACG GGC AAA GTG TGG CAG CTG GAC TAT	480
	Asp Ser Gly Thr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr	
	145 150 155 160	
	GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA AAA GCT	516
	Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala	
	165 170	

## 35 (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 172 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

## 40 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

	Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile
	1 5 10 15
45	Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe
	20 25 30

	Glu	Val	Ser	Ser	Thr	Lys	Trp	Phe	His	Asn	Gly	Ser	Leu	Ser	Glu	Glu
			35					40					45			
	Thr	Asn	Ser	Ser	Leu	Asn	Ile	Val	Asn	Ala	Lys	Phe	Glu	Asp	Ser	Gly
		50					55					60				
5	Glu	Tyr	Lys	Cys	Gln	His	Gln	Gln	Val	Asn	Glu	Ser	Glu	Pro	Val	Tyr
	65					70					75					80
	Leu	Glu	Val	Phe	Ser	Asp	Trp	Leu	Leu	Leu	Gln	Ala	Ser	Ala	Glu	Val
					85					90					95	
10	Val	Met	Glu	Gly	Gln	Pro	Leu	Phe	Leu	Arg	Cys	His	Gly	Trp	Arg	Asn
				100					105					110		
	Trp	Asp	Val	Tyr	Lys	Val	Ile	Tyr	Tyr	Lys	Asp	Gly	Glu	Ala	Leu	Lys
			115					120					125			
	Tyr	Trp	Tyr	Glu	Asn	His	Asn	Ile	Ser	Ile	Thr	Asn	Ala	Thr	Val	Glu
		130					135					140				
15	Asp	Ser	Gly	Thr	Tyr	Tyr	Cys	Thr	Gly	Lys	Val	Trp	Gln	Leu	Asp	Tyr
	145					150					155					160
	Glu	Ser	Glu	Pro	Leu	Asn	Ile	Thr	Val	Ile	Lys	Ala				
					165					170						